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WHAT ARE ENZYMES?

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THE word enzyme comes from a Greek word meaning "in yeast" (*en,* in; *zyme,* leaven). Perhaps the most acceptable definition in the light of recent scientific research is to say that it is a substance showing the properties of a catalyst and produced as a result of cellular activity.

But what is a catalyst? The reader will recall his first very simple experiment in the preparation of oxygen. Here the learned instructor tells the bewildered youth that if you put a little potassium chlorate in a test tube and heat this very strongly, a gas is evolved which is later identified as oxygen. Now by merely adding a small quantity of a dirty black-looking powder, called manganese dioxide, to the potassium chlorate, the oxygen is evolved much more rapidly and at a much lower temperature. But this is not all. A careful examination at the end of the reaction shows that the manganese dioxide has not changed in any way: we have the same substance, and the same amount, at the end of the reaction as at the beginning. Many such substances are known to chemists. They all have this peculiarity: that they *accelerate* chemical reactions, and that a relatively small—at times insignificant—quantity of the catalyst suffices to bring about the chemical change.

In cells we find substances of this type, but thus far these cellular catalysts, unlike the manganese dioxide, and like proteins, have never been produced outside of the cell.

When we consider that life is possible only because of continued cellular activity, and when we bear in mind that this activity is largely the result of chemical changes brought about by these enzymes, the paramount importance of these substances becomes manifest.

Alcoholic fermentation in yeast, the souring of milk, processes of putrefaction, and various other examples of changes in organic materials with, often enough, the accompanying liberation of bubbles of gas, had long been known. The epoch-making researches of Pasteur had shown that fermentations and putrefactions were inaugurated by the presence of living organisms. Then later extracts from the saliva and the gastric mucosa of the stomach were obtained which also had the power of bringing about chemical changes in carbohydrates and proteins. This led to the classification of ferments into those which, like yeast and certain bacteria, acted because of certain vital processes (organized ferments), and those which, like the extracts from the saliva and stomach, were presumably "non-living unorganized substances of a chemical nature" (unorganized ferments). Kühne designated the latter "enzymes." This classification was generally accepted, and the "vitalists" held absolute sway until Emil Buchner, in 1897, overthrew the whole theory by a series of researches which, in their influence, were only second in importance to those of Pasteur in an earlier generation. One of Buchner's classical experiments consisted in grinding yeast cells with sand and infusorial earth, and then subjecting the finely pulverized material to a pressure of 300 atmospheres—a pressure far more than enough to destroy yeast, or any other cells. The liquid so obtained had all the fermentative properties of the living yeast cell. Obviously, then, the living cell could not be responsible for the fermentation. On the other hand, this experiment did suggest that cellular activity gives rise to some substance which, once produced, exerts its influence whether the cell is alive or dead. All subsequent experiments have but strengthened the conviction that cells do produce these substances, and that the chemical changes are due *not* to the living organisms, but to the *lifeless* substances (enzymes) to which these organisms give rise.

Minute in quantity, and tenaciously adhering to substances present, particularly protein, the isolation of an enzyme in the pure state has become one of the most difficult problems in physiological chemistry. Yet any elementary student in the subject finds little difficulty in performing simple experiments which convince him either of its presence or of its absence. How are they done?

The method consists essentially in making use of the so-called "specificity" of enzymes. To use Fischer's simile, just as one key fits one lock, so any one enzyme will act on only a certain type of substance. Take, for example, the enzyme found in saliva, ptyalin: it readily acts on the carbohydrate, starch, but has absolutely no action on protein. Again, take the pepsin of the stomach: this enzyme breaks down proteins, but is without result on carbohydrates. These instances may be multiplied indefinitely.

Some enzymes show their specificity to an even more marked degree. In the yeast cell, for example, we find one, sucrase,[1] which acts only on cane sugar (sucrose) ; but on no other sugar or carbohydrate. A simple little experiment demonstrates this beyond question. A yeast cake is ground up very intimately with a little sand and water, and the mass filtered. A small portion of the filtrate is added to a solution of cane sugar, the mixture placed in an incubator kept at 38° C., and allowed to remain there for about 30 minutes or so. At the end of that time the mixture, if heated with Fehling's solution,[2] will yield a red-brick precipitate—a result which could not be obtained either with the cane sugar, or with the enzyme solution alone. No other carbohydrate solution—or protein, or fat solution, for that matter—can take the place of the cane sugar; our enzyme will be without effect. If we take our original yeast extract, and first heat it to, say, the boling point of water, then cool it, and from here on repeat the experiment as before, no grape sugar is obtained. If instead of heating the enzyme solution, we cool it, the action is considerably delayed.

Some of the yeast extract may be poured into an excess of alcohol, the precipitate separated by filtration, and redissolved in water. This solution will show all the properties of the yeast extract.

Evidently, then, the watery extract of yeast contains something which has the power of breaking down cane sugar. This something is exceedingly sensitive to heat, rather less so to cold, and is precipitated—together with other substances (as could be shown)—by alcohol. The last three properties are characteristic not only of sucrase, but of all enzymes to a greater or less degree. That a minute quantity of enzyme can act upon an exceedingly large quantity of substrate is also readily demonstrable. The laws of catalysis hold firm.

One other fact about enzymes is most important. Graham, as far back as 1861, found that certain substances (cane sugar, salt, etc.) in solution, when placed in a dialyzer consisting of a parchment bag, which in its turn was surrounded by water, would diffuse through the bag, whereas others (proteins, gum, starch, etc.) would not. The diffusible ones he named crystalloids, those non-diffusible, colloids. If some of our original yeast extract were placed in such a parchment bag, none of the enzyme would find its way into the surrounding layer of water. Enzymes, like the proteins, are *colloids*.

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[1] The ending "ase" denotes enzyme.

[2] This is the well-known alkaline copper solution used by all medical men to test for sugar in the urine. The sugar in the urine is not, as might be supposed, ordinary cane sugar, but grape sugar. Fehling's solution reacts with the latter, but not with the former.

The cane sugar is split or "hydrolyzed," by the sucrase, one of the products being grape sugar.

The fact that enzymes show colloidal properties, and the fact that they are invariably associated with proteins, made it seem probable that when ultimately isolated in the pure condition, they would be found to be proteins. Attempts to obtain pure enzymes have been many. The general method of procedure in almost all cases consists in first extracting with water[3] —as already explained—or submitting the mass to much pressure (Buchner), if the enzymes are "intracellular."

Having obtained a solution, the next step is often that of dialysis (Graham). Diffusible bodies, particularly inorganic substances, are thereby separated. Three of the classical investigators in this branch, Osborne, Peckelharing, and Fraenkel, have all employed this method.

Now usually comes precipitation. Some substance—alcohol, acetone, or ammonium sulphate—is added in which the enzyme is insoluble. The precipitate so obtained contains many impurities (proteins, certain carbohydrates, etc.). To purify it, it is redissolved, re-dialyzed, and reprecipitated many times. On occasion, a biological procedure, first suggested by Effront, and put into practise by Fraenkel, may be used. This consists in fermenting the impure precipitate with yeast. The carbohydrate and protein are thereby used up, but according to Fraenkel, the enzyme is not touched.

The laboriousness of such an operation may best be gathered from a specific example. Let us take an experiment from the work of Professor Sherman, of Columbia, an active investigator. Here is his method for preparing a starch-splitting enzyme from the pancreas: Mix thoroughly 20 grammes of pancreative powder—a commercial preparation—with 200 cubic centimeters of 50 per cent. alcohol at 15–20° C. [S. finds that much of the contained protein is left behind by the use of this 50 per cent. alcohol.] Allow this preparation to stand 5–10 minutes, then filter, keeping the temperature below 20° C. (This takes from 1 to 2 hours.) Pour the filtrate into 7 times its volume of a mixture of 1 part of alcohol to 4 parts ether (more protein and other impurities are here separated). Within 10–15 minutes the enzyme (including certain impurities) separates as an oily solution. Decant the supernatant liquid. Dissolve the precipitate in the smallest amount of pure water at a temperature of 10–15 degrees Centigrade and reprecipitate at once by pouring into 5 volumes of absolute alcohol. Allow it to settle, keeping temperature low; filter, dissolve in 200–250 cubic centimeters of 50 per cent. alcohol containing 5 grammes of maltose. Pour the solution into a collodion sack of 500 cubic centimeters capacity, and dialyze against 2,000 cubic centimeters of 50 per cent. alcohol at not above 20° C. and preferably not below 15° C. Replace dialyzate twice: after 15 hours and a second period of 8–9 hours with fresh 50 per cent. alcohol. Continue dialysis 40–42 hours. Filter. Pour clear filtrate into an equal volume of a mixture of alcohol and ether (equal parts). Filter in the cold, and place the precipitate in a vacuum desiccator.[4] The powder obtained is so active that it can digest 20,000 times its own weight of starch. And still we are not at all certain that this is an enzyme uncontaminated with foreign bodies!

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[3] Often containing alcohol, toluene, or chloroform (as preservatives).

Of the three or four representative workers in attempts to isolate a pure enzyme, the substances obtained by Professor Sherman and Dr. Osborne (of the Connecticut experiment station) showed decided protein characteristics; whereas the two German investigators, Lentner and Fraenkel, both agree in proclaiming their products as carbohydrate in nature. How near or how far from the truth is either group? To begin with, no proof that any of these products is 100 per cent. pure has been advanced, and the chemist through bitter experience knows the danger in discussing the composition of impure substances. Another fact to be kept in mind is that, often enough, the purer the enzyme, the less active does it become. In several of these cases it has been shown that a loss in activity goes hand in hand with a proportional loss in the phosphoric acid content of the substance. This gives rise to the possibility—expounded further on—that the enzyme is not a chemical individual, but consists of at least two substances: (*a*) a something which has the power of acting only when activated—in this instance—by (*b*) phosphoric acid. And yet, if arguing by analogy is at all permissible, it may be maintained that since all the inorganic catalysts are distinct chemical individuals, why not enzymes?

Of course, all this does not at all exclude the possibility that different enzymes may have different structures and the conflicting results of investigators may be due to this fact. Some of the men worked on amylases (starch-splitting enzymes), others on lipases (fat-splitting), others still on proteases (protein-splitting). Why assume that such diverse substances should all have the identical composition? It may be, as Professor Armstrong has suggested, that the enzyme in constitution is similar to the substance on which it acts.

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[4] A vessel (containing a hygroscopic substance to take up moisture) from which the air has been exhausted.

Extremely suggestive as the basis for much present-day activity has been the work of Professor Gabriel Bertrand, of the Sorbonne, Paris. Most of this has been on laccase, an oxidizing enzyme first found in the milky latex of the tree *Rhus vernicifera,* and since then in many plants. The production of the beautiful Japanese lacquer from the latex of *Rhus vernicifera* was shown to be due to the activation of the atmospheric oxygen by the laccase (hence its name). Bertrand was able to prove that the activity of the laccase was connected with the manganese present, for by repeated precipitation with alcohol, he divided his laccase preparations into three fractions of different manganese content, each with an activity distinctly proportional to the amount of manganese present. As further proof of the importance of this manganese, he was able to show that a minute addition of a salt of manganese (manganese sulphate) increased the activity of the laccase, whereas other metals had no such effect. This led him to the dual conception of an enzyme, also advocated by Armstrong: one of the constituents is capable of producing, to a slight degree, on its own account, the chemical reaction associated with the particular enzyme in question, but requires its activity to be augmented by the presence of another substance—inactive in itself—before its action becomes appreciable. The former may consist of acid, alkali, calcium or magnesium salt, etc. The latter component is more complex, usually protein-like (egg-white, for example), and colloidal.

Bertrand's views—perhaps, also, Fischer's colossal work on the synthesis of proteins from amino acids—has led the school of enzyme chemistry to shift its ground considerably. Why these laborious, and always futile attempts to isolate a pure enzyme from the cell? Why not attempt to synthesize one from simple inorganic and organic materials? Trillat, in 1904, prepared a mixture of traces of manganese chloride and egg albumen which showed the reaction of laccase and other oxidases (oxidizing enzymes): it blued guaiacum, its action was prevented by heat and acid, and it could be precipitated by alcohol, and redissolved in water without losing its oxidizing powers—characteristic properties of *all* enzymes. Wolf with his colloidal iron compounds, and Euler and Bolin with their calcium salts of organic acids (citric, malic, etc.), and many others, have produced strong evidence in favor of the view that many of the enzymes, at least many of the so-called oxidases, are relatively simple substances.

Along somewhat modified lines is the work of Panzer, who claims that various carbohydrates show distinct diastatic (carbohydrate-splitting) activity when heated with hydrochloric acid gas, and then ammonia; and that of Woker, whose findings, at present rather disputed, would tend to the belief that formaldehyde (the "formalin" of commerce) may, under certain conditions, act in place of diastase in hydrolyzing starch.

Some very far-reaching possibilities are suggested by the studies on the lipases (fat-splitting enzymes) of castor and soya beans by Dr. Falk, of the Harriman Research Laboratory. Every worker in the field is aware how very easily enzymes are inactivated or destroyed by heat or the presence of relatively small quantities of certain foreign bodies, such as acids and bases. The inactivation of the lipases of the beans could be brought about not only by these means, but also by neutral salts, alcohols, acetone, etc. Dr. Falk conceived the idea that this inactivation was due to an internal rearrangement of certain of the atoms in the molecule of the enzyme. Many cases of such tautomeric changes—of rearrangement within the molecule—are known to organic chemists, and are often stimulated by the action of mild chemical agents. Dr. Falk's hypothesis is to the effect that the grouping involved is to be found in all proteins, and hence, probably, in enzymes. If inactivation means the rearrangement of a group from configuration 1 to that of 2, activation, or change from 2 back to 1, may be brought about by the action of dilute alkali—often used to bring about these changes in configuration. Actual experiments on the action of alkali on proteins (themselves quite inactive) have endowed these substances with fat-splitting power.

Whilst, therefore, we are far from a comprehensive knowledge of the chemical configuration of an enzyme, studies on the production of artificial enzymes, and on the possible rearrangements of certain groups within the molecule, may throw much light on a very perplexing problem.